

Effects of Polybrominated Biphenyls on Kidney Function and Activity of Renal Microsomal Enzymes

by K. M. McCormack,* W. M. Kluwe,* V. L. Sanger,†
and J. B. Hook*

Polybrominated biphenyls (PBBs) cause hepatic microsomal enzyme stimulation and histopathological alterations in several organs, including kidney. Concern about effects of PBBs on the health of newborns has increased after the discovery of PBBs in milk of nursing mothers. Therefore, it was of interest to investigate the effects of PBBs on kidney function and the activity of renal microsomal enzymes in adult and immature animals. Seven and eleven day old pups were treated with a single IP injection of either peanut oil or 150 mg/kg PBBs (FireMaster BP-6) in peanut oil. Adult virgin rats were fed diet containing 0 or 100 ppm PBBs for 30 or 90 days. Treatment with PBBs only retarded weight gain after 90 days exposure. Kidney-to-body weight ratio was not altered by PBBs. Arylhydrocarbon hydroxylase activity was increased while epoxide hydratase activity was decreased (adults) or not affected (immature rats) in kidney following treatment with PBBs. Administration of PBBs had no effect on blood urea nitrogen, the clearance of inulin, *p*-aminohippurate (PAH), or fractional sodium excretion. Similarly, the *in vitro* accumulation of PAH and *N*-methylnicotinamide (NMN) by thin renal cortical slices and ammoniogenesis and gluconeogenesis in renal cortical slices were not affected by PBBs. In conclusion, treatment with PBBs resulted in modification of the activity of renal microsomal enzyme activities but had no detectable effect on renal function.

Introduction

Polybrominated biphenyls (PBBs) are fire retardants which contaminated livestock and poultry feed in Michigan. The commercial product blended with feed was FireMaster BP-6, which is a mixture of PBB congeners; the major component is 2,2',4,4',5,5'-hexabromobiphenyl (1). Rats maintained on diet containing PBBs have enlarged livers as well as hepatic and renal histopathological alterations (2-4). Renal lesions observed following treatment with 100 ppm octabromobiphenyl include petechial hemorrhage and hyaline degenerative cytoplasmic changes (2, 3). Rat urinary protein is elevated after subchronic administration of PBBs (5). These observations suggest that renal function may be impaired by PBBs.

When administered acutely or subchronically, PBBs increase the activity of hepatic drug metabolizing enzymes in rats (6, 7). Stimulation of hepatic microsomal enzymes following PBBs is similar to that observed after treatment with both phenobarbital (PB) and 3-methylcholanthrene (3MC) (6), two compounds which represent cytochrome P-450 and P₁-450 inducing agents, respectively (8). Currently, there is a scarcity of information regarding PBBs effects on the activity of extrahepatic microsomal enzymes. Increased activity of mixed function oxygenases may alter the metabolism of endogenous compounds and xenobiotics.

Concern about potential effects of PBBs on mothers and offspring has been generated since high concentrations of PBBs have been detected in human milk (9). Young animals may be particularly vulnerable to damage caused by PBBs. The kidney is morphologically and functionally immature at birth and deficient in its capacity to maintain homeostasis when challenged with stressful stimuli. Therefore, it was of interest to investigate the ef-

* Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824.

† Department of Pathology, Michigan State University, East Lansing, Michigan 48824.

fects of PBBs on kidney function and the activity of renal microsomal enzymes in adult and immature animals.

Methods

Adult, female virgin and lactating Sprague-Dawley rats and pups (10 animals per litter) (Spartan Farms, Haslett, Mich.) were maintained in clear polypropylene cages at 22°C with a 12-hr light cycle (7 AM-7 PM) and were allowed free access to food (Wayne Lab Blox) and water. Animals were acclimatized for at least 3 days prior to experimental treatment. The 7- and 11-day old pups were treated with a single IP injection of either peanut oil (10 ml/kg) or 150 mg/kg PBBs (FireMaster BP-6, Michigan Chemical Corp., St. Louis, Michigan) in peanut oil, 10 ml/kg. Following acclimation, adult virgin rats were treated by substituting ground diet containing 0 or 100 ppm PBBs (FireMaster BP-6) for the Lab Blox. Rats were exposed to the diet for 30 or 90 days.

Enzyme assays were performed 28 days following injection of seven day old pups and after 90 days exposure of adult rats to the experimental diet. Immediately following sacrifice, kidneys were excised, weighed and then chopped into ice-cold 66mM Tris buffered to pH 7.4 with HCl. Renal postmitochondrial supernatants were prepared by homogenization (Potter-Elvehjem homogenizer with a Teflon pestle) in 3 volumes of 66mM Tris pH 7.4 followed by centrifugation at 10,000g for 30 min. All assays were performed on the day of supernatant preparation. Protein was measured (10) by use of bovine serum albumin as a standard. Enzyme activities measured were epoxide hydratase (EH) (11) and arylhydrocarbon hydroxylase (AHH) (12, 13).

Animals used for histologic examination had been exposed to the experimental diet for 90 days. Pieces of kidney were cut and fixed in 10% buffered formalin. After fixation, these were embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin.

Organic ion transport capacity was estimated *in vitro* 3 days following injection of 11-day-old pups and after 30 and 90 days exposure of adult rats to the experimental diet. Following sacrifice, kidneys were quickly removed, weighed, and placed in ice-cold normal saline. Transport capacity was quantified as the ability of renal cortical slices to accumulate a representative anion, *p*-aminohippuric acid (PAH), and cation, *N*-methylnicotinamide (NMN) (14, 15). Results were expressed as slice-to-medium (*S/M*) concentration ratio, where *S* is in units of mg/g wet tissue weight and *M* is expressed in mg/ml medium. The ability of renal cortical slices

from rats treated with PBBs for 90 days to form ammonia and glucose was also determined (16, 17). Net production of ammonia and glucose was expressed as μ mole/mg wet tissue weight/hr.

To determine renal function *in vivo*, animals exposed to PBBs for 30 or 90 days were anesthetized with 50 mg/kg sodium pentobarbital, IP, and body temperature was maintained at 36–38°C by use of heat lamps. A PE50 cannula was inserted into the bladder, and urine was collected under mineral oil in preweighed vials. The left femoral vein was cannulated for infusion. Both femoral arteries were cannulated to monitor blood pressure and to obtain blood samples. The infusion solution contained 1% inulin and 0.6% PAH in normal saline. (3 H)-Inulin (0.5 μ Ci/ml) and (14 C)-PAH (0.5 μ Ci/ml) were added to the solution, which was infused at 0.019 ml/min using a Harvard infusion pump. A minimum of 1.5 hr elapsed from the beginning of the infusion to initiation of urine collection. The 30-min urine collections were taken with blood (400 μ l) sampled at the middle of each period. (14 C)-PAH, (3 H)-inulin, and sodium concentrations were determined as previously described (18). Blood urea nitrogen (BUN) was quantified (17) and expressed as weight (mg) urea nitrogen per 100 ml of whole blood.

Data were analyzed statistically by randomized complete block analysis of variance. Treatment differences were detected by the least significant difference test (19). The 0.05 level of probability was used as the criterion of significance.

Results

Growth rate was not affected by a single IP injection of 150 ppm (150 mg/kg) PBBs or by 30 days treatment with 100 ppm PBBs; however, 90 days dietary exposure to 100 ppm PBBs retarded weight gain (Table 1). The kidney to body weight ratio was not altered by treatment with PBBs (Table 1).

Table 1. Effect of PBBs on body weight and kidney to body weight ratio.

Treatment time, days	PBB Treatment, ppm	Body weight, g ^a	Kidney weight/body weight ^a
30	Control	234.2 \pm 6.9	0.78 \pm 0.07
	100	221.5 \pm 8.3	0.75 \pm 0.04
90	Control	276.7 \pm 4.8	0.71 \pm 0.02
	100	250.2 \pm 5.4 ^b	0.76 \pm 0.04
Acute ^c	Control	121.4 \pm 1.3	1.05 \pm 0.04
	150	117.2 \pm 0.7	1.03 \pm 0.08

^a Values are means \pm S.E.M. for four animals.

^b Statistically significant difference from the respective control, *p* < 0.05.

^c Seven-day-old rats were given a single treatment with PBBs and sacrificed 28 days later.

Administration of 150 ppm PBBs to 7-day-old rats increased the activity of renal arylhydrocarbon hydroxylase (AHH) above controls 28 days later (54% of control). Epoxide hydratase (EH) activity was not modified in kidneys of young rats by PBBs. The activity of renal AHH in rats exposed to 100 ppm PBBs for 90 days was increased above control (1116% of control), however, renal EH activity was significantly decreased (14% of control) (Table 2).

Table 2. Effect of PBBs on renal AHH and EH activities.

Treatment time, days	PBB treatment, ppm	AHH, fluorescence units/mg microsomal protein-min ^a	EH, nmole styreneglycol formed/mg microsomal protein-min ^a
90	Control	2.94 ± 1.35	0.14 ± 0.06
	100	32.82 ± 1.73 ^b	0.02 ± 0.01 ^b
Acute ^c	Control	4.39 ± 2.36	0.29 ± 0.02
	150	24.08 ± 8.22 ^b	0.33 ± 0.03

^a Values are means ± S.E.M. for three animals.

^b Statistically significant difference from the respective control, $p < 0.05$.

^c Seven-day-old rats were given a single treatment with PBBs and sacrificed 28 days later.

Degenerative histopathological alterations were observed in kidney from rats treated with 100 ppm PBBs for 90 days (Fig. 1). Renal changes included progressive obsolescence of glomeruli. Glomerular tufts were shrunken. Bowman's membrane, while not thickened, was diminished in proportion to the shrinking tuft. A single focus of lymphocytes was seen in one kidney.



FIGURE 1. Renal tissue from rat fed diet containing 100 ppm PBBs for 90 days. There are shrunken glomeruli. Hematoxylin and eosin, 133×.

Treatment with PBBs had no effect on the *in vitro* accumulation of PAH and NMN by thin renal cortical slices (Table 3) or ammoniogenesis and gluconeogenesis by renal slices (Table 4).

The clearance of inulin (glomerular filtration rate, GFR), the clearance of PAH (effective renal plasma flow, ERPF) and fractional sodium excretion (%) were unaffected by 30 or 90 days exposure to diet containing 100 ppm PBBs (Table 5). Similarly, administration of PBBs for 30 or 90 days had no effect on blood urea nitrogen (BUN) (Table 6).

Table 3. Effect of PBBs on the accumulation of PAH and NMN.

PBB treatment, ppm	S/M ratio ^a	
	PAH	NMN
30 Days	Control	10.17 ± 1.46
	100	9.84 ± 0.87
90 Days	Control	10.49 ± 0.43
	100	10.16 ± 0.76
Acute ^b	Control	8.29 ± 0.81
	150	8.56 ± 0.33

^a Values are means ± S.E.M. for at least three animals.

^b Two-week-old rats were given single treatment with PBBs 72 hr prior to sacrifice.

Table 4. Effect of feeding PBBs for 90 days on ammoniogenesis and gluconeogenesis.

PBB treatment, ppm	Net production, $\mu\text{mole/mg wet tissue weight/hr}^a$	
	Ammonia	Glucose
Control	3.39 ± 0.09	0.03 ± 0.01
100	3.45 ± 0.09	0.03 ± 0.01

^a Values are means ± S.E.M. for four animals.

Discussion

Polybrominated biphenyls stimulate hepatic microsomal mixed function oxygenases in adult rats and mice (6, 7, 20). The results of this investigation indicate that PBBs also alter the activity of renal microsomal enzymes in immature and adult rats. Activity of AHH is increased in kidney as in liver after PBBs; however, in contrast to hepatic EH stimulation, renal EH activity is decreased (adults) or not affected (immature rats) by treatment with PBBs. Stimulation of AHH with concomitant inhibition of EH in kidney may be of toxicological concern. Microsomal enzymes such as AHH can catalyze the formation of very reactive arene oxides from inert aromatic compounds (21, 22). Arene oxides may be further metabolized to less toxic dihydrodiols by enzymes such as EH (22). Thus, since AHH activity is increased while EH activity is

Table 5. Effect of feeding PBBs on glomerular filtration rate (GRF), effective renal plasma flow (ERPF), and fractional sodium excretion.

Treatment time, days	GFR, ml/min ^a		ERPF, ml/min ^a		Fractional sodium excretion, %	
	Control	100 ppm PBBs	Control	100 ppm PBBs	Control	100 ppm PBBs
30	2.78 ± 0.19	2.42 ± 0.57	9.47 ± 0.54	9.01 ± 0.32	0.14 ± 0.06	0.29 ± 0.11
90	2.46 ± 0.16	2.34 ± 0.21	10.87 ± 0.63	8.77 ± 0.97	0.17 ± 0.04	0.12 ± 0.03

^a Values are means ± S.E.M. for four animals.

Table 6. Effect of feeding PBBs on blood urea nitrogen.

PBB treatment, ppm	Duration, days	BUN, mg urea nitrogen/100 ml/blood ^a
Control	30	17.28 ± 2.75
100	30	22.19 ± 1.92
Control	90	19.06 ± 1.86
100	90	18.59 ± 1.52

^a Values are means ± S.E.M. for four animals.

decreased (adults) or not affected (immature rats) in kidney following treatment with PBBs, subsequent administration of a compound metabolized by these enzymes may result in increased concentrations of arene oxides and possibly amplified nephrotoxicity.

Weight gain was only retarded following 90 days exposure to 100 ppm PBBs. Previous reports suggest that this may be due to decreased food efficiency (5, 23). Kidney enlargement was not detected and although glomerular degenerative alterations were observed following treatment with PBBs for 90 days, similar changes were seen in controls but less frequently. Since more severe renal degenerative changes were seen following treatment with 100 ppm octabromobiphenyl (2, 3), FireMaster BP-6, which is mostly hexabromobiphenyl (1), may be less nephrotoxic. The paucity of prominent renal histopathological alterations after exposure to FireMaster BP-6 correlates with our observations regarding its lack of effect on renal function.

Exposure to 100 ppm PBBs for 30 or 90 days had no effect on BUN consistent with its lack of effect on GFR. Similarly, ERPF, filtration fraction (GFR/ERPF) and fractional sodium excretion were unaffected by PBBs. Treatment with PBBs had no detectable effect on any *in vitro* parameters of renal function tested.

Acute and subchronic administration of PBBs to immature and adult rats, respectively, modified the activity of renal microsomal enzymes. Alterations in enzyme activities were not correlated with changes in renal function. This indicates that PBBs, at least FireMaster BP-6, may not be a potent nephrotoxic agent. However, the characteristics of mixed function oxygenase alteration in kidney fol-

lowing treatment with PBBs suggest that this compound may predispose the kidney to toxicity produced by chemicals administered subsequent to PBBs.

This investigation was supported in part by the USPHS grants ES00560 and GM 01761. We wish to express our appreciation to C. Herrmann and L. Lepper for their excellent technical assistance. We also wish to thank D. Hummel for preparation of the manuscript.

REFERENCES

1. Sundström, G., Hutzinger, O., and Safe, S. Identification of 2,2',4,4',5,5'-hexabromobiphenyl as the major component of flame retardant FireMaster BP-6. *Chemosphere* 5: 11 (1976).
2. Aftmosis, J. G., Culik, R., and Lu, K. P. Toxicology of brominated biphenyls. I. Oral toxicity and embryotoxicity. *Toxicol. Appl. Pharmacol.* 22: 316 (1972).
3. Norris, J. M., et al. Toxicological and environmental factors involved in the selection of decabromodiphenyl oxide as a fire retardant chemical. *J. Fire Flammability/Combustion Toxicol.* 1: 42 (1974).
4. Corbett, T. H., et al. A toxicity of polybrominated biphenyls (FireMaster BP-6) in rodents. *Environ. Res.* 10: 390 (1975).
5. Sleight, S. D., and Sanger, V. L. Pathologic feature of polybrominated biphenyl toxicosis in the rat and guinea pig. *J. Amer. Vet. Med. Assoc.* 169: 1231 (1976).
6. Dent, J. G., Netter, K. J., and Gibson, J. E. The induction of hepatic microsomal metabolism in rats following acute administration of a mixture of polybrominated biphenyls. *Toxicol. Appl. Pharmacol.* 38: 237 (1976).
7. Dent, J. G., et al. Induction of hepatic microsomal metabolism by a mixture of polybrominated biphenyls. *J. Toxicol. Environ. Health* 3: 651 (1977).
8. Sladek, N. E., and Mannering, G. J. Induction of drug metabolism. I. Differences in the mechanism by which polycyclic hydrocarbons and phenobarbital produce their inductive effects on microsomal N-demethylating systems. *Mol. Pharmacol.* 5: 174 (1969).
9. DHEW Report. Final report of the subcommittee on the health effects of polychlorinated biphenyls and polybrominated biphenyls. Department of Health, Education and Welfare, Washington, D. C., 1976.
10. Lowry, O. H., et al. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265 (1951).
11. Oesch, F., Jerina, D. M., and Daley, J. A radiometric assay for hepatic epoxide hydratase activity with [7-³H] styrene oxide. *Biochim. Biophys. Acta* 227: 685 (1971).
12. Nebert, D. W., and Gelboin, H. V. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. *J. Biol. Chem.* 243: 6242 (1968).

13. Oesch, F. Differential control of rat microsomal "Aryl hydrocarbon" monooxygenase and epoxide hydratase. *J. Biol. Chem.* 251: 79 (1976).
14. Cross, R. J., and Taggart, J. V. Renal tubular transport: Accumulation of *p*-aminohippurate by rabbit kidney slices. *Amer. J. Physiol.* 161: 181 (1950).
15. Smith, H. W., et al. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J. Clin. Invest.* 24: 388 (1945).
16. Roobol, A., and Alleyne, G. A. O. Control of renal cortex ammoniogenesis and its relationship to renal cortex gluconeogenesis. *Biochim. Biophys. Acta* 353: 83 (1974).
17. Kaplan, A. Urea nitrogen and urinary ammonia. In: *Standard Methods in Clinical Chemistry*. S. Meites, Ed. Academic Press, New York, 1965.
18. Pegg, D. G., et al. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on renal function in the rat. *J. Toxicol. Environ. Health* 2: 55 (1976).
19. Steel, R. G. D., and Torrie, J. H. *Principles and Procedures of Statistics*. McGraw-Hill, New York, 1960.
20. Farber, T. M., and Baker, A. Microsomal enzyme induction by hexabromobiphenyl. *Toxicol. Appl. Pharmacol.* 29: 102 (1974).
21. Daley, J. W., Jerina, D. M., and Witkop, B. Arene oxides and the NIH shift: The metabolism, toxicity and carcinogenicity of aromatic compounds. *Experientia* 28: 1129 (1972).
22. Oesch, F. Mammalian epoxide hydrolases: Inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. *Xenobiotica* 3: 305 (1973).
23. Garthoff, L. H., et al. Biochemical and cytogenetic effects caused by ingestion of Arochlor 1254 or FireMaster BP-6. *J. Toxicol. Environ. Health* 3: 769 (1977).